



AN EVALUATION OF TWO METHODS FOR THE IDENTIFICATION
OF FAECAL STREPTOCOCCI.

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Taxonomy Section

Bacteriology Branch
DIVISION OF LABORATORIES

May, 1971

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An Evaluation of Two Methods for the Identification of Faecal Streptococci.

Introduction

Two methods were tested in seeking a scheme for the rapid identification of Group D Streptococci. The cultures tested were isolated colonies taken from membrane filter - Enterococcus plates supplied by the River and Sewage laboratories.

Methods and Materials

Method #1:

A representative number of colonies were picked from each plate and each colony was inoculated into azide dextrose - modified (AD(M))broth (4). The broths were incubated at 35°C for 48 hours. Negative tubes were discarded. Inocula from the positive tubes were transferred to ethyl violet azide (EVA) broth tubes. These tubes were incubated for 48 hours. In this method, all broths and agars used were incubated at 35°C. Positive EVA tubes were streaked on tetrazolium glucose agar (TZA) plates (1), which were incubated primarily for 48 hours. If there was insufficient growth to perform the tests, the plates were left for an additional 24 hours. Each culture was Gram stained and examined microscopically.

The colonial growth was divided into two types. Intensely deep red colonies with a white periphery were called Type I, and white or pale pink colonies without any periphery were called Type II.

Type I colonies were tested for gelatin liquefaction, hemolysis using 15% Human and 5% Horse blood agar, starch hydrolysis, growth on potassium tellurite agar, and fermentation reactions in Melibiose and Sorbitol using a 1% carbohydrate concentration in purple broth base. Carbohydrate reactions were recorded between 24 and 72 hours.

The Type II colonies were tested for fermentation reactions in Arabinose, Glycerol, Melezitose, Raffinose and Sorbitol carbohydrates in purple broth base, and for starch hydrolysis and growth on potassium tellurite agar.

The above method was a modification of the scheme used by Raj, H., W.J. Wiebe and J. Liston (4). Our modification involved the use of 5% Horse blood, and the tests for starch hydrolysis and growth on potassium tellurite agar.

Method #2:

The cultures were obtained from the same sources as for Method #1. The colonies were inoculated into AD(M) and EVA broths, and streaked on TZA plates as in the previous method. Only the pink to red (Type I) colonies were picked from these plates. A confirmation for catalase negativity was done on these colonies. White and very pale pink colonies were not tested. The catalase negative cultures were inoculated into two tubes of brain heart infusion (BHI) broth, and one tube of 40% bile broth. One tube of

BHI broth was incubated at 45°C for two days, and the other at 10°C for 5 days; the bile broth was incubated at 20°C for 48 hours. The tubes were checked for growth during, and at the end of the incubation periods.

Tubes with growth at 45°C and 10°C were then inoculated in 6.5% NaCl in BHI broth for confirmation. If positive, the culture was presumed to be Lancefield Group D Streptococci. It was then tested for starch hydrolysis: if positive, the classification was Atypical S. faecalis; if negative, litmus milk was inoculated for peptonization. If the litmus milk was peptonized, the culture was classified as S. faecalis var. liquefaciens. A negative litmus milk reaction classified the organism as an Enterococci.

Cultures with growth at 45°C only, were assumed to be S. bovis or S. equinus. These organisms were tested for the ability to hydrolize starch. If this test result was positive then the fermentative reaction of lactose in purple broth base was determined. Acidity of the lactose classified the culture as S. bovis while a no change reaction indicated S. equinus.

After the primary checking for growth at 45°C and 10°C, all other tests were incubated at 20°C. The choice of temperature was governed by the assumed temperature of the sample when it was collected, as recommended by Geldreich and Kenner (3).

Results

In Method #1, 710 colonies were picked from the MF-Enterococcus plates. Six isolates failed to grow in AD (M) broth, five in EVA broth, and twenty-five on TZA agar. The remaining 674 cultures were classified as follows:

Type I :	166	<u>S. faecalis</u>
	4	<u>S. faecalis</u> var. liquefaciens
	29	Unclassified type.
Type II:	166	<u>S. faecium</u>
	74	<u>S. durans</u>
	2	<u>S. bovis</u>
	233	Unclassified type.

In Method #2, 50 colonies were picked from Enterococcus plates. All cultures were taken to the TZA stage, but the project was interrupted and only 18 cultures had final tests done for classification. These 18 cultures were tested in parallel with Method #1. With Method #1, all 18 cultures were classified as S. faecalis, and as Enterococci with Method #2.

Discussion

"When TZA medium of Barnes is added as a third step after the EVA procedure, a rapid and accurate estimation of faecal contamination may thus be obtained. However, it may be noted that TZA medium by itself is not selective for enterococci. Organisms other than Enterococci can grow and produce colonies if not screened by the AD(M), EVA steps. Therefore, the use of TZA medium for direct counting of enterococci in a sample would be unsatisfactory and erratic, unless it is made by adding some inhibitory agent, such as thallous acetate" (4). No false positives were encountered in the cultures tested.

Use of the modified Azide Dextrose broth was very advantageous. The addition of the indicator (0.003% brom-thymol blue) made the results very clear out. A negative reaction was a slight colour change to pale green, and a positive, a change from greenish blue to bright yellow. All cultures tested were bright yellow in AD(M) broth.

Haemolysis on the Horse blood agar was better defined than on the Human blood agar. However, there were certain disadvantages connected with the use of Horse blood, particularly the cost and the inconvenience of obtaining it. Human blood was easily obtained from the Red Cross without charge.

Method #2 was the scheme outlined by Geldreich and Kenner (3). In this paper there were points of method not clearly explained by the authors.

There was no mention of the incubation period and temperature for the preliminary test for growth in 40% bile, and for the confirmatory test (growth in 6.5% NaCl in BHI). Growth at 45°C only identified S. bovis or S. equinus. Cultures which were positive for starch hydrolysis were then tested for lactose fermentation. No mention was made of those cultures which were negative for starch hydrolysis and these occurred quite frequently.

The authors pointed out the need for a revised definition of the enterococci (3). They used the classification enterococci to denote organisms from warm blooded animal sources, exclusive of S. bovis and S. equinus. Had their scheme not avoided mention of S. faecalis, this grouping would have been acceptable. The omission of the typical S. faecalis group insinuated that these organisms were included in the newly defined group "enterococci". Furthermore, the fact that S. faecalis var. liquefaciens merited a distinct classification indicated that this organism was a separate species, in contrast to currently accepted belief (2).

Their distinction between Lancefield Group D and S. faecalis (Atypical and liquefaciens varieties) excluded the latter from Lancefield Group D. Since it was not stated whether or not the Lancefield grouping classification was confirmed by sera typing, this distinction was questionable.

Conclusion and Recommendation

Presently, in routine analyses, white colonies on membrane filters are not counted as Streptococci. In Method #1, white colonies were tested and found to belong to the Group D Streptococci. It is quite possible that by ignoring these colonies, the counts so generated are erroneous. Perhaps this is an area that requires more investigation and possibly revision.

Of the two methods tested, Method #1 is definitely the better scheme for the rapid identification of faecal streptococci. The time factor involved in Method #2 would be a disadvantage, if laboratory reports depended on the results of the tests. There is a preliminary 5 day waiting period for growth at 10°C. Added to that is the time needed for the completion of the tests. It would be approximately two weeks before a report could be sent out.

With the increasing awareness of faecal streptococci as a general index of faecal contamination, and of S. faecalis as a specific index, it would be interesting to apply Method #1 or some other reliable method to a study of the occurrence and strain distribution of faecal streptococci in waters of a specific area.

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An evaluation of two
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